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The Effect of K⁺ on Caspase Activity of Corneal Epithelial Cells Exposed to UVB

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Abstract

Exposure of human corneal limbal epithelial (HCLE) cells to UVB triggers rapid loss of K⁺ and apoptosis via activation of caspases-9, -8 and -3. It has been shown that preventing loss of intracellular K⁺ can inhibit apoptosis. The goal of this study was to investigate the effect of K⁺ on the UVB-induced caspase activity. HCLE cells were exposed to 150 mJ/cm² UVB, followed by measurement of caspase activity in cell lysates. Caspase activity was measured in the presence and absence of 100 mM K⁺ in the reaction buffer. UVB-induced activity of caspases-9, -8 and -3 all decreased in the presence of 100 mM K⁺. These results suggest that a role of high [K⁺] in the cell is to inhibit caspase activity. Therefore, when cells lose K⁺ in response to UVB, caspases are activated and cells go into apoptosis. This supports our hypothesis that K⁺ inhibits caspase activity.

We have published several reports showing that apoptosis of human corneal limbal epithelial (HCLE) cells can be inhibited if loss of intracellular K⁺ in response to exposure to UVB radiation is prevented (Singleton et al., 2009; Ubels et al., 2010, 2016; Glupker et al., 2016). The mechanism by which the relatively high concentration of intracellular K⁺ inhibits apoptosis in these cells has not been investigated. Apoptosis, which is caused by a variety of factors, including UV radiation, usually begins with the activation of K⁺ channels and the loss of intracellular K⁺ (Bortner et al., 1997, 2007; Yu et al., 1997; Lu et al., 2003; Wang et al., 2004). As discussed in more detail below, the loss of K⁺ appears to cause activation of caspases, specifically caspases -9, -8, and -3, which serve an essential role in the execution of apoptosis. Caspases -8 and -9 are apoptosis initiators, while caspase-3 is an apoptosis effector enzyme (Inoue et al., 2009).

These caspases are activated in response to UV radiation in many different cell types. Caspase-3 activity was reported to increase when human myeloblastic cells were exposed to UVB (Arrebola et al., 2006). UVC also increases caspase-3 and caspase-8 activity in Jurkat T-cells (Vu et al., 2001). UVC and UVB radiation increased caspase-3 activity in rabbit corneal epithelial cells (Lu et al., 2003) and a human corneal epithelial cell line (Shimmura

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et al., 2004), respectively, as well as in keratinocytes (Daher et al., 2006). Finally, we have shown that HCLE cells exposed to UVB at doses relevant to ambient outdoor exposure show increased caspase-9, caspase-8, and caspase-3 activity (Singleton et al., 2009; Glupker et al., 2016).

It has been suggested in previous studies that elevated intracellular $[K^+]$ prevents activation of apoptotic pathways (Hughes et al., 1997; Thompson et al., 2001). Exposure of HCLE cells to 150 mJ/cm² UVB activates K^+ channels, allowing for efflux of intracellular K^+ , resulting in apoptosis (Singleton et al., 2009; Ubels et al., 2011). After UVB exposure, incubating HCLE cells in medium with elevated extracellular $[K^+]$ (25–100 mM) as compared to the usual $[K^+]$ of 5 mM in culture medium, or with the K^+ channel blocker, Ba²⁺, prevents UVB-induced loss of K^+ from the cells (Ubels et al., 2011; Glupker et al., 2016), which in turn significantly decreases the activation of caspase-3, caspase-8, and caspase-9 by UVB (Singleton et al., 2009; Glupker et al., 2016).

The purpose of the present study was to further investigate the effect of K^+ on the activity of caspase-9, caspase-8, and caspase-3 after UVB exposure. Our hypothesis was that addition of K^+ to the reaction medium would inhibit caspase activity in lysates of HCLE cells that had been exposed to UVB.

HCLE cells were grown to confluence in 6-well plates in Keratinocyte Serum-Free Media (KSFM, Life Technologies, Grand Island, NY). The cells were exposed to 150 mJ/cm² UVB, incubated in KSFM for 6 hours, collected and lysed as previously described (Singleton et al., 2009). Protein in the cell lysates was measured by the Bio-Rad assay (Berkeley, California). Caspase activity (relative fluorescence units / mg protein) was measured using fluorometric caspase-9, caspase-8, and caspase-3 assay kits (Biovision, Milpitas, California), with modifications described below. It is important to note that all solutions in the caspase kits contained no K^+ .

A saturated KCl solution (4.4 M in water) was prepared, and 24.1 μ L of this solution was added to 12.0 μ L of deionized water and 463.9 μ L of reaction buffer. This resulted in a K^+ concentration of 210 mM. To initiate the caspase assay, a 50 μ L aliquot of this solution and 5 μ L of DEVD-AFC substrate were added to 50 μ L of cell lysate, achieving a final K^+ concentration of 100 mM. The samples were then incubated at 37° for two hours and caspase activity was measured using a fluorescence microplate reader.

UVB-induced caspase-3 activity significantly decreased, from 994.7 RFU/mg protein in the reaction buffer without K^+ to 862.4 RFU/mg protein in buffer containing 100 mM K^+ (Fig. 1). Caspase-8 activity also decreased significantly in the presence of 100 mM K^+ . UVB-induced activity decreased from 587.1 RFU/mg protein in the samples without K^+ to 424.7 RFU/mg protein in the samples containing high $[K^+]$. UVB-induced caspase-9 activity decreased significantly as well, from 244.1 RFU/mg protein in buffer without K^+ to 133.3 RFU/mg protein in the samples containing 100 mM K^+ . The data show that high K^+ concentrations inhibit UVB-induced caspase activity, suggesting that high intracellular K^+ may inhibit apoptosis by suppressing the activity of caspases.

It is possible, however, that the inhibition of the caspases by addition of KCl to the reaction buffer was caused by the increase in osmolarity of the solution. In order to test this, we measured caspase-3 activity in the presence of increased osmolarity of the reaction buffer using sucrose rather than potassium. A saturated sucrose solution was prepared by adding 10 g sucrose to 5 mL of deionized water. A 24.1 μ L aliquot of the sucrose solution was added to the reaction buffer as described above. This achieved a final sucrose concentration of about 133 mM and the same final osmolarity in the assay system, measured with a vapor pressure osmometer (Wescor, Logan, UT), as when KCl was added to the buffer. UVB-induced caspase-3 activity was measured as described above.

The data indicate that raising the osmolarity of the reaction system with sucrose had no effect on caspase-3 activity. The UVB-induced caspase-3 activity in the reaction buffer containing no sucrose was 939.2 RFU/mg protein, while caspase-3 activity in the reaction mixture containing sucrose was 934.9 RFU/mg protein (Fig. 2). Therefore, the decrease in caspase activity in the above experiments is due to increased $[K^+]$.

Our observation that 100 mM K^+ inhibits UVB-induced caspase activity is in contrast to a report by Hughes et al. (1997), who used dexamethasone to induce apoptosis in thymocytes. When they added 100 mM K^+ to the reaction buffer, they observed no effect on caspase-3 activity that had been induced by exposure of the cells to dexamethasone. However, when they induced thymocyte caspase-3 activity *in vitro* using dATP and cytochrome C, high $[K^+]$ in the reaction buffer inhibited the caspase activation.

Although we did detect inhibition by K^+ of UVB-activated caspases *in vitro*, the inhibition of caspase-3 was relatively low at 13.3%. However, 100 mM K^+ inhibited caspase-8 by 27.7%, and caspase-9 by 45.4%. Since caspase-3 is downstream from caspase-9 and caspase-8, this means that inhibition of caspase-9 and caspase-8 by intracellular K^+ could have a relatively strong effect on the effector caspase-3 in the intact cell. The inhibitory effect of high $[K^+]$ on caspase-9 *in vitro* agrees with our recent report showing that the intrinsic pathway dominates the effect of UVB on apoptosis of HCLE cells (Ubels et al., 2016).

This study supports our overall hypothesis that the relatively high $[K^+]$ in tears reduces the electrochemical gradient for loss of intracellular K^+ in corneal epithelial cells in response to UVB (Singleton et al., 2009; Ubels et al., 2011, 2016; Glupker et al., 2016). This may contribute to protection of the corneal epithelium from ambient UVB by inhibiting apoptotic pathways that are normally suppressed by the relatively high intracellular K^+ concentration.

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Figure 1.

Effect of high [K⁺] on UVB-activated caspase activity after exposure of HCLE cells to 150 mJ/cm² UVB. Incubation of cell lysates with 100 mM K⁺ caused a significant decrease in UVB-induced activity of all three caspases (mean $\pm$ SD, t-test, n=12, p = 0.05).

Figure 2. Raising the osmolarity of the reaction medium using sucrose rather than KCl had no effect on caspase-3 activity (mean $\pm$ SD, t-test, n=12, p = 0.05).